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GRAFTING AND REINCORPORATION IN ACTINOSPHÆRIUM EICHHORNII EHR.

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It is a well known fact that contiguous individuals [1] in a culture of *Actinosphærium eichhornii* may fuse along the surfaces in contact. Cytoplasmic union of this type, or plastogamy,[2] has been frequently described, and has often been considered as an important initial step in the evolution of nuclear union.

In the closely allied form *Actinophrys sol,* plastogamy is a preliminary step toward nuclear fusion. A striking photomicrograph of this process, taken from living material, is shown by Bělař, '22, (Taf. 3, Fig. 24). Fusion of a number of these individuals around a large food mass, has recently been described by Looper, '25. This union is temporary, for separation occurs as soon as digestion is completed.

Attempts to produce plastogamous fusion artificially have met with varying degrees of success. Cienkowski, '65, was able to fuse two vegetative masses of *Actinosphærium* if he brought together two cut surfaces, and held them together with paper strips. Johnson, '94, could not bring about artificial coalescence in a single instance. Greff, '67, and Penard, '04, observed that a single vegetative mass, if fragmented into a large number of portions under a cover slip, might eventually fuse into the original form. In some cases, this may have been a simple contraction phenomenon, for no attempt was made to break apart the cytoplasmic threads connecting the crushed fragments.

[1] In behavior, the single sphere commonly termed an individual *Actinosphærium,* shows all the characteristics of a colonial aggregate. This would seem the more accurate terminology to apply to each vegetative mass.

[2] Also spelled plasmogamy. Bělař, '22.

In the course of an extended physiological study of Actinosphærium eichhornii, it was observed that artificial plastogamy could invariably be induced by suspending two or more animals in a hanging drop small enough to exert moderate compression. Various types of simple grafts were found to be successful when sufficient pressure was exerted by this method to enforce temporary contact.

Also, the interesting behavior of axopods [3] which were occasionally severed from the main body during the transfer of animals to hanging drops, seemed worthy of critical attention. Reincorporation of pseudopodial fragments has been reported in a few instances. Jensen, '96, and '01, describes this phenomenon in *Orbitolites* and *Amphistegina*. Kepner and Reynolds, '23, observed in *Difflugia* the recovery of separated pseudopodial fragments by their cell bodies.

The results derived from these accidental and experimental graftings, and from the severing of axopods, in *Actinosphaærium* are presented in the present paper.

Material and Methods.

Actinosphærium eichhornii was reared in large numbers in a medium containing ciliates, rotifers and round worms. Subcultures were made in Great Bear Spring Water, to which had been added concentrations of *Colpoda, Paramecium, Noteus* and *Philodina*.

Gross merotomy was performed under a binocular microscope. To aid in differentiating between the cut portions of two individuals, half of the animals to be grafted were stained in a very dilute solution of neutral red, made up in the original medium. The neutral red penetrated easily, and stained the cytoplasmic granules a deep red. A stained (red) and an unstained (white) animal were placed in a Syracuse watch glass, and cut into halves or quarters with a glass hair. Before normal contraction could cause rounding of these portions, two or more were transferred to

[3] Greeff (*loc. cit.*) saw "unipolar, pear-shaped cells," among the fragments resulting from his crushing experiments. In all probability, these were derived from axopodia, but their origin could not be accurately observed when so drastic a method of separation was applied.

a hanging drop, the size of which was reduced until the animals came into contact at the desired angle. These were transferred to a moist chamber for observation under high power (Leitz oc. 8x, obj. 7a).

Severing of individual axopodia was accomplished by means of the Chambers micrurgical apparatus, and the subsequent behavior followed under high power.

Results.

(*a*) *Grafting of Cut Portions.*

Grafted animals fused perfectly in all cases. The portions taken from each of the two contributing animals remained distinctly marked off. The surfaces held in contact varied in position and extent.

Record 3, (Text-figures 1 to 7), covers briefly the range of possible variations. In Figs. 1 and 2, two cut medullary surfaces (*M–M*) were brought into contact. Two quadrants of a stained individual brought into contact, medulla to medulla, (*M–M*), and medulla to cortex, (*m–C*), are shown in Figs. 3, 4 and 5. Figs. 6 and 7 represent a case in which an entire stained (red) animal was grafted on to half of an unstained (white) individual.

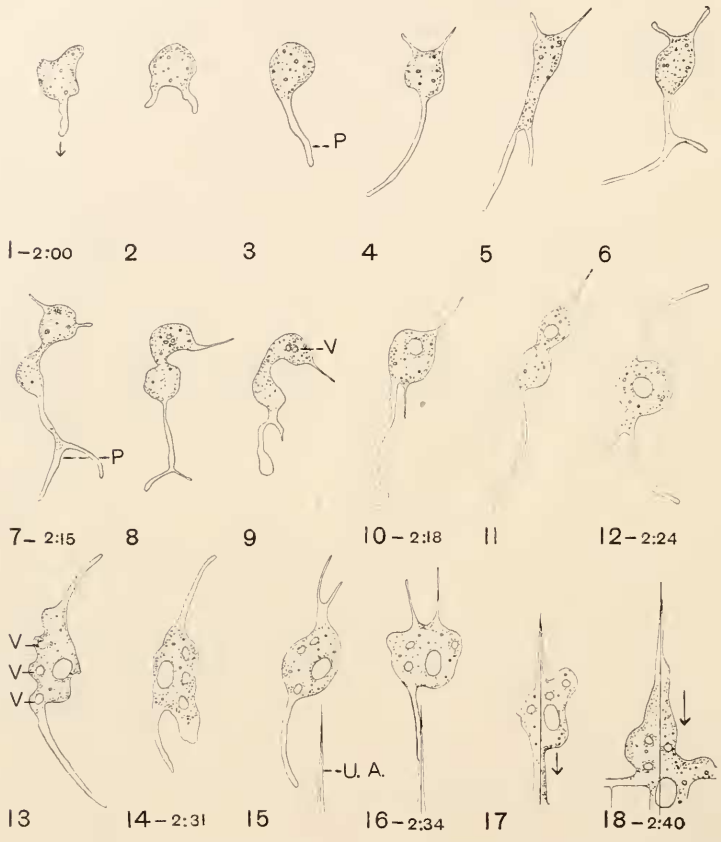
The time taken for complete fusion of surfaces varied from five to twenty minutes. As the animals were returned to the deeper water in a Syracuse watch glass, they resumed their normal spherical shape.

The extent and position of the grafted portions was still recognizable at the end of thirty hours.

(*b*) *Merotomy and Reincorporation of Axopodia.*

Axopodia were severed from the main body, carried away by the needle for some distance to break all connecting strands of protoplasm, and returned to the immediate region of the animal. Freed from the microneedle, they exhibited slow, erratic, swinging movements. If these movements brought them into chance contact with an unsevered axopod, or with the cortical surface of the main body, they were incorporated (Records 1, 2 and 5).

The first step after severance is usually the rounding up of the broad basal end of the axopod as an irregular granular mass, into which the extended portion is gradually withdrawn (Record 4; Figs. 1, 2, 3), and from which new pseudopodial extensions of

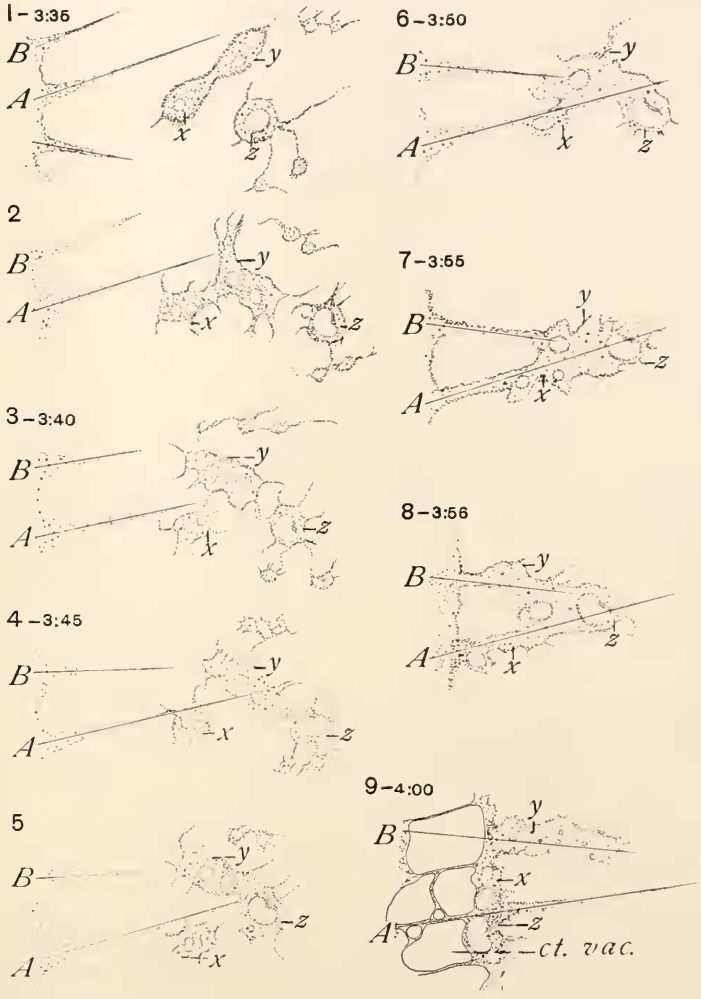


Record 1, Figs. 1–18. Changes of form exhibited by a severed axopod before reincorporation. Time lapse, 2:00–2:40. *P*, pseudopodial extensions. *V*, newly formed vacuoles. *U. A.*, axopod still unsevered from the main body.

great length are thrown out (Fig. 4). During its migration, this body becomes increasingly vacuolated (Record 1, Figs. 10–18). No axial cores were ever observed in its pseudopodia (filopodia).

A number of severed axopodia may unite to form a loose network (Record 2, Figs. 1 to 9), which behaves as a single unit.

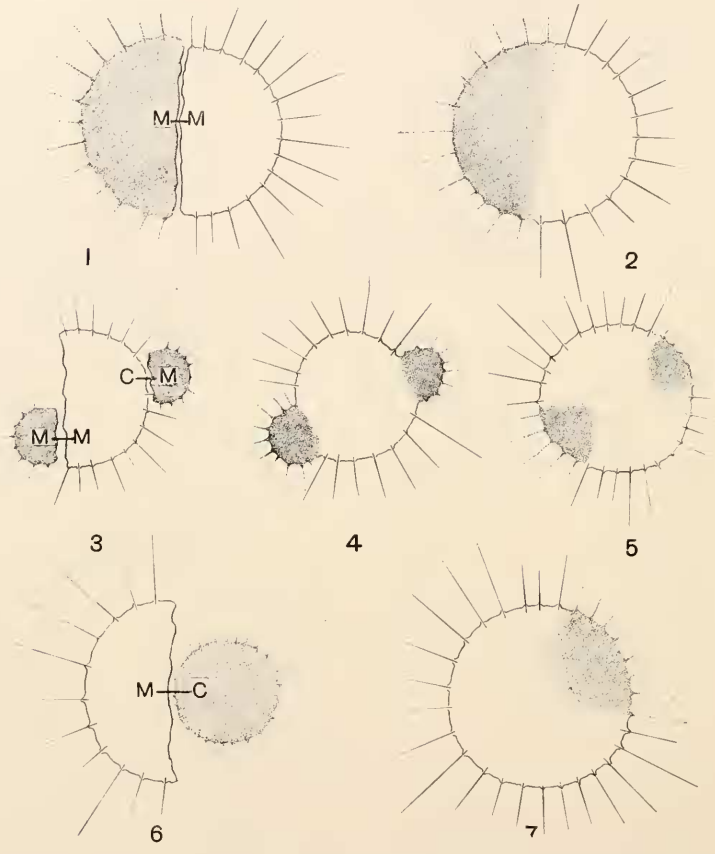
The first sign of protoplasmic union may occur at either the tip or the side of an axopod. Fragments reincorporated near the



Record 2. Figs. 1–9. Protoplasmic network formed from a number of severed axopodia. *x*, *y*, *z*, individual axopodia, gradually fusing, and finally reincorporated as a single mass. Time lapse, 3:35 to 4:00. *A* and *B*, the two unsevered axopodia chiefly concerned in the reincorporation process. *Ct. vac.*, vacuoles of the cortical layer of the main body.

tip, increase the size of an axopod at that point to several times the original diameter, and have the appearance of a large lateral

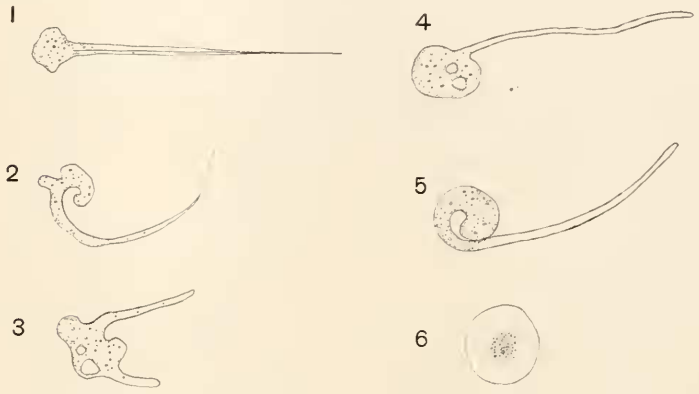
swelling (Record 5, Fig. 6). This mass travels centripetally, flattening as it goes, and fuses completely at the level of the cortical surface (Record 5, Fig. 7; Record 1, Fig. 18).



Record 3, Figs. 1–7. Diagrammatic representations of different types of grafting between stained and unstained vegetative masses of *Actinosphærium eichhornii*. Stippled areas represent portions of animals stained with dilute neutral red. Clear areas represent portions of unstained animals (white). *M–M*, medulla grafted to medulla. *C–M*, cortex grafted to medulla.

Axopodia cut from one animal and carried on the needle point to the immediate region of another, will be incorporated if the protoplasm is still capable of locomotion. Dead (motionless) axopodial masses are refused.

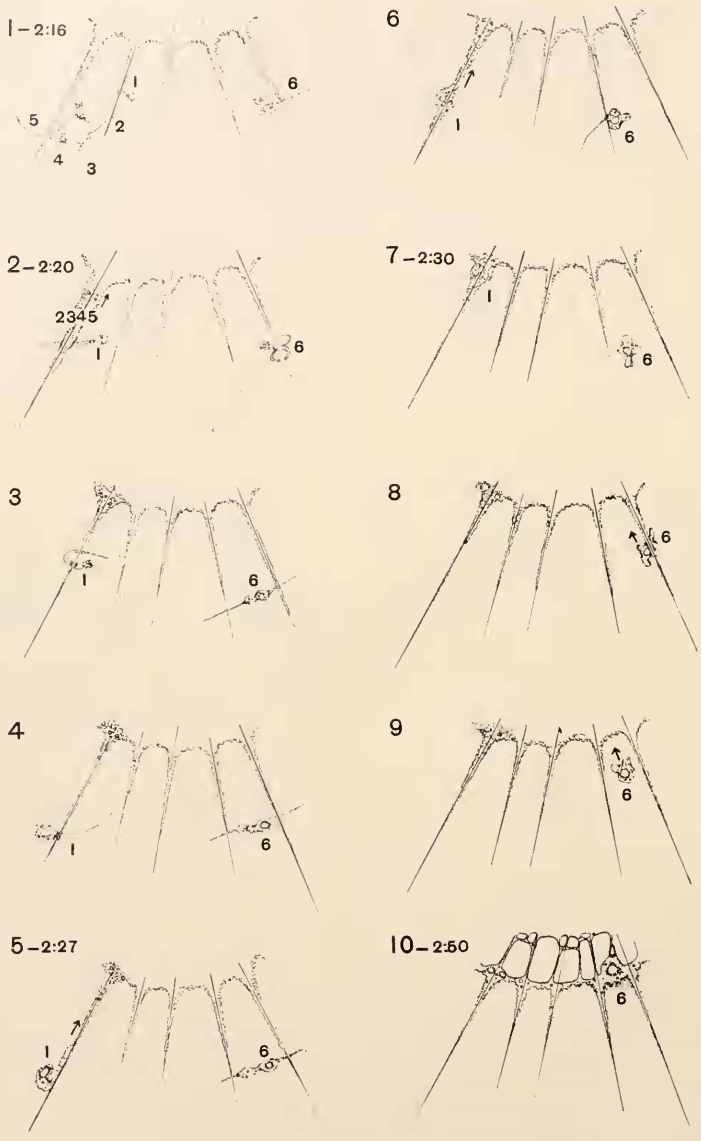
Frequently, severed axopodia were observed which approached very closely to an animal without fusing, and subsequently moved away. If fusion had not occurred at the end of 40 to 50 minutes, the pieces became spherical and quiescent, and showed signs of hyalinization and disintegration (Record 4, Fig. 6).



Record 4. Figure 1 represents an axopod just severed from the main body. Figs. 2 and 3, stages during resorption of the original axopodial extension. Fig. 4, newly formed pseudopod, without axial core. Fig. 5, twisting movement which results in a clumsy locomotion. Fig. 6, hyalinization and disintegration after failure to become reincorporated.

It is demonstrated in the foregoing experiments that the highly vacuolated cytoplasm of the vegetative masses (colonial aggregates) of *Actinosphærium* is very adhesive. The ease with which artificial plastogamy or grafting is accomplished under compression, strongly suggests the possibility that the failure of Johnson, '94, to fuse vegetative individuals was due to the lack of some similar device. Also, the essential factor in the experiments of Cienkowski, '65, would seem to be the compression which he applied by the use of paper strips, rather than the approximation of two cut surfaces.

Reincorporation of severed axopodia is, in reality, the same process on an infinitely smaller scale. The fusion, in these cases, is unlike reincorporation in *Difflugia* in that it may occur at the tip or side of an unsevered axopod, or at the cortical surface of the main body.



RECORD 5. Figs. 1–10. Diagrammatic representation of the process of reincorporation of six severed axopodia, numerically labelled. Time lapse for reincorporation of numbers 2, 3, 4 and 5, was four minutes; for number 1, fourteen minutes. Number 6 did not fuse with unsevered axopodia, but moved slowly toward the cortex, fusing there in thirty-four minutes.

Summary.

Grafting.

1. Merotomized portions of *Actinosphærium eichhornii* may be grafted together under slight compression.

2. Fusion occurs between two medullary, or between medullary and cortical surfaces.

3. Grafted animals regain a spherical shape, and show no sign of separation at the end of four days.

Reincorporation of Axopodia.

1. Axopodia completely severed from the protoplasmic mass may be reincorporated, either by the same or by another animal.

2. Severed axopodia often become vacuolated, and develop long pseudopodia.

3. Reincorporation may occur at either the tip or the sides of unsevered axopodia, or on the cortical surface of the main body.

4. Severed axopodia which have not been reincorporated at the end of 40 to 50 minutes, round up, become quiescent, and show signs of disintegration.

5. Motionless axopodial fragments are not incorporated.

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